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LABORATORY TECHNIQUES FOR REARING HELIOTHIS SPECIES ON ARTIFICIAL MEDIUM

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Laboratory studies of the bollworm (Heliothis zea (Boddie)) and the tobacco budworm (H. virescens (Fabricius)) have been limited to a great extent because of the lack of satisfactory rearing methods. Techniques used to rear the bollworm and the tobacco budworm for 18 and 5 consecutive generations, respectively, on a laboratory-prepared artificial diet with no outside stock are described in this report.

The diet differs only slightly from that described by Vanderzant et al.2/ It is prepared in batches of approximately 1 gallon in a large Waring blender.3/ The order of adding the ingredients is arranged to obtain a good solution of soluble materials and to minimize the exposure of labile compounds to strong alkali and prolonged high temperature. Distilled water is used unless another solvent is indicated. The ingredients for one batch of diet in the order in which they are added to the blender are as follows:

1/ In cooperation with the Texas Agricultural Experiment Station.

2/ Vanderzant, E. S., Richardson, C. D., and Fort, S. W., Jr. Rearing of the bollworm on artificial diet. Jour. Econ. Ent. 55: 140. 1962.

3/ Reference to commercial products and companies in this report does not imply their endorsement by the U.S. Department of Agriculture over similar products and companies not named.

Material	Amount
Water	880 ml.
22.5-percent potassium hydroxide	18 ml.
Casein, vitamin free	126 g.
Salt mixture, Wesson's	36 g.
Sucrose	126 g.
10-percent formaldehyde	13 ml.
15-percent methyl parahydroxybenzoate in 95-percent ethyl alcohol	36 ml.
10-percent choline chloride	36 ml.
Wheat germ	108 g.
Alphacel	18 g.
Vitamin suspension[1/]	6 ml.
Agar, hot, dissolved in 2,200 ml. of boiling water	90 g.
Ascorbic acid	14.4 g.
Aureomycin	500 mg.

The ingredients for this diet can be obtained from most biochemical suppliers. Its preparation can be simplified by using a commercially prepared vitamin diet-fortification mixture. One satisfactory mixture is available from the Nutritional Biochemicals Corporation, Cleveland 28, Ohio. Thirty-six g. of this NBC mixture will replace the choline chloride and the vitamin mixture included in the preceding list of ingredients. If the NBC mixture is used, the amount of sucrose should be reduced to 96 g. per batch of diet, since the vitamins are triturated in dextrose. About 13 g. of ascorbic acid per batch should be added, since the amount in the NBC mixture is not sufficient.

The contents of the blender are mixed intermittently as the ingredients are added and are thoroughly mixed for 2 to 3 minutes after all the materials are added. Also, short periods of mixing are desirable while dispensing the diet to retain uniformity of that part still in the blender. Approximately 15 to 20 g. of diet is poured into each 8-dram shell vial. Flexible polyethylene mustard- or catsup-dispensing bottles are useful for filling these vials. Then the vials are set aside until the excess moisture evaporates.

1/ Contains the following ingredients in milligrams per milliliter of suspension: Niacin 6, calcium pantothenate 6, riboflavin 3, thiamine hydrochloride 1.5, folic acid 1.5, pyridoxine hydrochloride 1.5, biotin 0.12, and vitamin B_{12} 0.012.

The insect eggs are collected on cheesecloth. They are surface sterilized to minimize the spread of disease by immersing the cheesecloth in 0.15-percent sodium hypochlorite for about 5 minutes. The hypochlorite is neutralized with sodium thiosulfate, and the cloth and eggs are rinsed with distilled water. Eggs that are washed off the cheesecloth are collected on a filter in a Büchner funnel. The wet cheesecloth is hung to drip-dry and then placed, along with the eggs on the filter, in jars covered with paper toweling.

The eggs hatch in 3 to 4 days at about 75° F. One newly hatched larva is transferred to each 8-dram rearing vial. The vials are plugged with cotton and placed in a continuously lighted, windowless room maintained at about 80°. Pupae are obtained in approximately 2 weeks. The pupae are removed, washed in hypochlorite in the same manner as the eggs, and placed in 1-gallon widemouthed glass jars on lightly moistened vermiculite. The jars are covered with cheesecloth and a strip of cheesecloth is draped into the jar so that the emerging moths can climb up to a hanging position and expand their wings. The pupae, as well as the adults, are held in a daylight room maintained at about 80° with windows on the south, west, and north sides.

A pioneer-size lantern globe is used as an oviposition chamber. The globe is set on a $6\frac{1}{2}$-inch milk filter disk, which covers moist sand in a pie pan. A piece of cheesecloth is draped into the lantern globe to provide a resting place for the moths, and the top is covered with cheesecloth, where most of the eggs are laid. A 150-mm. petri dish top is placed over the lantern globe to hold the cheesecloth in place and to maintain a high humidity in the container. Under extremely dry conditions a milk filter disk is cut to fit inside the inverted petri dish cover and wetted to provide additional moisture. High humidity appears to be essential for consistent oviposition. About 10 pairs of moths are placed in each oviposition chamber. The moths are fed a 5-percent sugar solution on a wad of cotton in a 50-mm. petri dish.

Larvae for toxicological investigations are reared in 20- by 150-mm. petri dishes on 75 to 100 g. of diet, which is cut into small pieces after it has hardened. Newly hatched larvae are placed in the dish, a milk filter disk is placed over the dish, and the cover is put in place. After 3 to 4 days, depending on the amount of moisture condensation in the dish, the cover is removed and a Masonite ring is used to hold the filter-disk cover in place. Approximately 50 to 75 third-instar larvae can be obtained from each dish in 5 days.

Considerable mold develops on the feces of the larvae held for pupal development in the 8-dram vials. Several antimicrobial agents have been added to the diet to try to alleviate this problem, but none have been found that will pass through the insect, remain effective, and not retard the development of the insect. Aureomycin is added to reduce the amount of yeast growth under conditions of high humidity, especially in the petri dishes where large numbers of larvae are reared together at one time.